METHODS FOR PRODUCING AN ACTIVE CONSTITUENT OF A PHARMACEUTICAL OR A DIAGNOSTIC AGENT IN AN MDCK CELL SUSPENSION CULTURE

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a National Stage application of co-pending PCT application PCT/EP02/10208 filed Sep. 11, 2002, which was published in German under PCT Article 21(2) on Mar. 20, 2003, which claims the benefit of German application Serial No. DE10144906.2 filed Sep. 12, 2001. These applications are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

The invention concerns methods for production of an active ingredient of a drug or diagnostic agent in which viruses are multiplied in MCDK (Mandine Darby canine kidney) cells on a commercial scale in suspension culture.

BACKGROUND OF THE INVENTION

Infectious diseases, especially viral infections, are still of major medical importance. The need to make available better methods by means of which viruses can be multiplied in culture in order to permit research on viruses and production of vaccines therefore remains unchanged. Production of vaccines, in particular, against viral infections ordinarily requires multiplication and isolation of large amounts of the corresponding virus.

Depending on the corresponding virus, different host systems and culture conditions for virus multiplication are used in the prior art. Standard host animals, embryonic chicken eggs, primary tissue cell cultures or established permanent cell lines are used as host systems (Rolle and Mayr (editors), Microbiology, Infection and Epidemic Science, 1978; Mahy (editor), Virology, A Practical Approach, 1985; Horzinek (editor), Compendium of General Virology, 1985).

Virus multiplication in embryonic chicken eggs is connected with high costs and time demands. The eggs must be incubated before infection and then tested for viability of the embryos. Only living embryos are capable of multiplying viruses. After infection with the virus being multiplied has occurred and further incubation, the embryos are finally killed. The viruses isolated from the egg are freed of contaminants and concentrated. Since multiplication of viruses in incubated eggs is not possible under strictly sterile conditions, contaminating pathogenic microorganisms must be eliminated from the isolates if these are to be available for medical or diagnostic application.

An alternative to multiplication of viruses in chicken eggs is offered by eukaryotic host cells of defined cell lines (Gregersen, J. P., Pharmazeutische Biotechnologie, Kayser and Muller (editors), 2000, pp. 257-281). Numerous cell lines, however, are not suitable for production of vaccines or similar medically useable preparations owing to persistent foreign virus contaminations or because of the absence of demonstration of freedom from viruses, unclear origin and history.

On the other hand, the Vero cells derived from the kidney cells of monkeys are a host system that is already being used in the multiplication of individual viruses (polio virus, rabies virus) for vaccine production. These cells are available in different cell banks (for example, the American Type Culture Collection, ATCC) and are also made available by the World Health Organization (WHO) from a tested cell bank for medical research.

These Vero cells are adherent lines that require support surfaces for their growth, like glass bottles, plastic culture plates or plastic flasks. Growth on so-called microcarriers occurs in a culture of corresponding cells in the fermenter, i.e., generally small plastic spheres on whose surface the cells can grow.

It is known that adherent BHK (baby hamster kidney) and adherent MDCK (Madin Darby canine kidney) cells and other cells can also actively multiply viruses, in addition to the aforementioned Vero cells, and are being used as substrate for production of pharmaceutical products or their use is being considered. In the MDCK cell line ATCC CRL34 (NBL-2), in addition to influenza viruses, the vesicular stomatitis virus, the Coxsackie virus B5 (but not B3 or B4), reovirus [sic; typo in German] types 2 and 3, adenovirus types 4 and 5, as well as vaccinia viruses have also been experimentally multiplied. All corresponding publications, however, are geared exclusively toward adherent cultures (cf. ATCC product information). However, the suspension culture is preferred for multiplication of larger cell amounts, in which only the lymphoid and many transformed cells could thus far be multiplied in this system (Lindl (editor), Cell and Tissue Culture, 2000, pp. 173 ff). An MDCK cell line that is able to grow in suspension in protein-free culture media is disclosed in WO 97/37000. Multiplication of influenza viruses using the corresponding host cells is also described.

In addition to selection of an appropriate cell or host system, the culture conditions under which a virus strain is multiplied are also of great significance for the achievement of an acceptably high yield. To maximize the yield of desired virus strains, both the host system and the culture conditions must therefore be specifically adapted in order to achieve favorable environmental conditions for the desired virus strain. In order to achieve a high yield of different virus strains, a system that creates optimal growth conditions is therefore required. Many viruses are restricted to special host systems, some of which are very inefficient with respect to virus yield. Efficient production systems are often based on adaptations of the virus population of corresponding culture systems, often using intermediate stages with other host systems and employing protein additives—mostly serum of animal or human origin.

It is also known to experienced persons that nearly all cell cultures, after initial multiplication with addition of serum or other growth factors, can be kept at least for a certain time without serum or protein additives. For example, an arbitrary cell culture can be transferred at the time of virus infection or right before harvesting to a medium without serum or protein additives and kept until harvest. This has been common practice for years in order to obtain virus material for vaccines or diagnostic tests, while avoiding or reducing foreign proteins. Vaccines and cell cultures that were kept without this practice during the infection phase with addition of serum will have greater problems in being allowed for use in humans or animals, since the serum components can scarcely be adequately eliminated (cf. WHO recommendations "Proposed requirements for measles vaccine" (Live), Requirements for Biological Substances No. 12, revised 1978).

It is also known that many viruses can only be multiplied very poorly or not at all in protein-containing media. Viruses that rely on the activity of proteolytic enzymes (proteases)